

# 5793

## Feasibility of using microbiological indicator assays to detect temperature abuse in refrigerated meat, poultry, and seafood products

*The feasibility of using commonly employed microbiological indicator tests to determine if a product has been temperature abused was evaluated in raw and ready-to-eat meat, poultry, and seafood products. Twelve retail products were purchased, subdivided into three subsamples, and stored at 5°C (adequate refrigeration), 12°C (moderate abuse), and 19°C (gross abuse). They were periodically sampled for aerobic plate counts (plates incubated at 28, 37, and 42°C), presumptive coliforms, confirmed coliforms, thermal tolerant coliforms, Escherichia coli, and Staphylococcus aureus. No single indicator test was effective in all of the foods examined, but different assays were able to differentiate temperature abused versus adequately refrigerated samples in a number of the products. The results suggest that the characterization of how different microbiological indicators respond to storage temperatures in various products may be an approach for developing more effective microbiological criteria for refrigerated foods of animal origin.*

### Introduction

Various microbiological indicator tests have been used extensively in food microbiology, based on the need for simple tests that assess the microbiological safety or quality of food products. However, the usefulness of these tests has been generally limited, in part because they are often employed for the wrong reasons. Many indicator tests were initially developed for other systems, and then adapted to foods. For example, the coliform test, originally proposed by

Schardinger and Theobald Smith in the 1890s, was developed as a means for assessing the likelihood that water supplies were contaminated with fecal material and thus a potential source of *Salmonella typhi*. The test has a number of underlying assumptions including: (1) coliform bacteria are ubiquitously present in fecal material and consistently present in higher numbers than *Salmonella*; (2) neither coliforms nor *Salmonella* will grow in water; and (3) the coliforms are more resistant to adverse conditions present in the aquatic environment than *Salmonella* and will become inactivated at a slower rate than the pathogen (Jay 1978). The second assumption is particularly important in relation to establishing the quantitative aspects of the assay, i.e.

the number of coliforms is proportional to the extent of fecal contamination.

The coliform test was adapted for use with pasteurized milk; however, it is important to note the underlying assumptions in this application are distinctly different than those for water. The key assumptions for pasteurized milk are that: (1) the indicator is at least as heat resistant as *Salmonella*; (2) normal pasteurization is more than adequate to eliminate coliforms from the product; (3) the primary source of coliforms in finished product is due to non-fecal, post-pasteurization contamination; and (4) the growth of the indicator organism is faster than the pathogen, particularly under conditions of temperature abuse. When these assumptions are considered it becomes apparent that the use of the coliform test with pasteurized milk is not a measure of fecal contamination, but instead is a measure of process integrity. Process integrity can be viewed as the integrated systems controls that are employed to prevent the growth of pathogenic micro-organisms (NAS 1985, Buchanan 1991). In the case of pasteurized milk, the three primary controls are thermal processing, prevention of post-pasteurization recontamination, and maintenance of adequate refrigerated storage.

After reviewing how various microbiological indicator tests are used to assess the microbiological quality or safety of raw and cooked, ready-to-eat meat, poultry, and seafood products, it became apparent that most of the tests are employed as process integrity indicators, particularly in relation to the adequacy of refrigerated storage conditions. However, there seems to have been little systematic assessment of the feasibility of using microbiological indicator tests for evaluating the likelihood that a product was temperature abused. One of the requirements for a good microbiological

indicator of temperature abuse would be that the level of the indicator remains unchanged or below a limit as long as the product was adequately refrigerated, but increases rapidly if the product was exposed to any significant temperature abuse. The objective of the current study was to determine the potential for using commonly employed microbiological indicator tests to identify refrigerated meat, poultry, and seafood products that had been temperature abused. The overall approach was to store identical portions of retail raw and ready-to-eat foods at temperatures corresponding to adequate refrigeration, moderate temperature abuse, and gross temperature abuse, periodically determining the quantitative status of eight indicator tests.

## Materials and Methods

### *Culture media*

All media were obtained from Difco Labs, Inc. (Detroit, MI) and prepared as specified by the manufacturer.

### *Food acquisition and preparation*

Foods were purchased from the meat, seafood, or delicatessen departments of local retail supermarkets on the day of each experimental run. The products were refrigerated until initiation of experimentation, with the elapsed time between purchase and the initial sampling being less than 1 h. The foods evaluated included cooked 'salad-size' shrimp, raw medium-size shrimp, seafood salad, unpasteurized lump crabmeat, raw ground beef, raw ground pork, sliced cooked ham, sliced roast beef, raw ground chicken, sliced chicken roll, sliced low-salt turkey breast, and chicken salad. Additional experimentation on the effects of elevating plate count incubation temperatures used cooked 'salad-size' and 'medium-size' shrimp and raw medium-sized shrimp. The foods were mixed thoroughly and transferred in three 120 g portions to sterile 500 ml beakers covered with aluminium foil. For ease of sampling, foods that were purchased as larger

pieces (e.g. sliced roast beef, medium-size shrimp, etc) were aseptically chopped up and mixed thoroughly prior to being transferred to the beakers. Immediately after removing the day 0 samples, one beaker of each food sample was incubated at 5, 12, and 19°C to simulate adequate refrigeration, moderate temperature abuse, and gross temperature abuse, respectively.

The sampling times were chosen to take into account the different growth rates associated with the three incubation temperatures, with the 5°C foods being sampled on days 0, 2, 3, 7, 10; the 12°C foods on days 0, 2, 3, 4, and 7; and the 19°C foods on days 0, 1, 2, 3, and 4. The contents of each beaker were mixed with a sterile spatula prior to removal of a 5.0 g ( $\pm$  0.2) sample. The sample was then homogenized with 45 ml of sterile 0.1% peptone water in a 'filter stomacher bag' using a Stomacher (Model 400, Tekmar, Inc.). The filtrate was collected, and diluted appropriately using sterile 0.1 peptone water.

#### *Microbiological testing*

At each sampling time, the foods were evaluated for aerobic plate counts (28, 37, and 42°C incubation), *Staphylococcus aureus*, presumptive coliforms, confirmed coliforms, thermal tolerant (fecal) coliforms (Buchanan 1991), and *Escherichia coli*, using relatively minor modifications of the procedures outlined in the FDA BAM Handbook (AOAC 1986). Aerobic plate counts (APC) were performed by plating six plates per dilution on prepared brain heart infusion agar (BHIA) plates using a spiral plater (Spiral System Instruments, Inc., Bethesda, MD). Duplicate plates were incubated at 28, 37, and 42°C for 24 h and enumerated using an automated colony counter (Model 500A, Spiral System Instruments, Inc.).

Presumptive *S. aureus* counts were determined by direct plating (using a spiral plater) on Baird-Parker agar (BPA) plates. The plates were incubated 24 h at 37°C, and colonies displaying the characteristic colonial morphology and tellurite-positive response enumerated. When no positive colonies were observed, the plates were incubated for an additional 24 h and re-examined.

The levels of coliforms, thermal tolerant coliforms, and *E. coli* were determined using a three-tube MPN (0.1, 0.01, 0.001, and 0.0001 g food) series. Presumptive coliforms were determined using lauryl sulfate tryptic

tose broth (37°C incubation). Positive tubes were used to inoculate brilliant green bile broth (37°C incubation) and EC broth (45.5°C incubation) to estimate confirmed coliforms and thermal tolerant coliforms, respectively. All tubes were incubated for 48 h and examined for positive turbidity and gas production after 24 and 48 h. *E. coli* was identified by subculturing EC tubes on Levine EMB agar plates for 48 h and then confirming the identity of presumptive colonies by performing IMViC tests.

#### *Supplemental elevated incubation temperature TAPC assays studies*

Cooked and raw shrimp were purchased from three different local supermarkets, prepared, incubated and sampled at intervals as described above. At each sampling, 10 g of food were mixed with 90 ml of diluent in a filter stomacher bag and homogenized for 2 min with a stomacher. Dilutions were prepared and plated on pre-poured BHIA plates using a spiral plater. Duplicate plates were incubated for 24 h at 28, 42, 45, and 48°C and then enumerated using the automated counter.

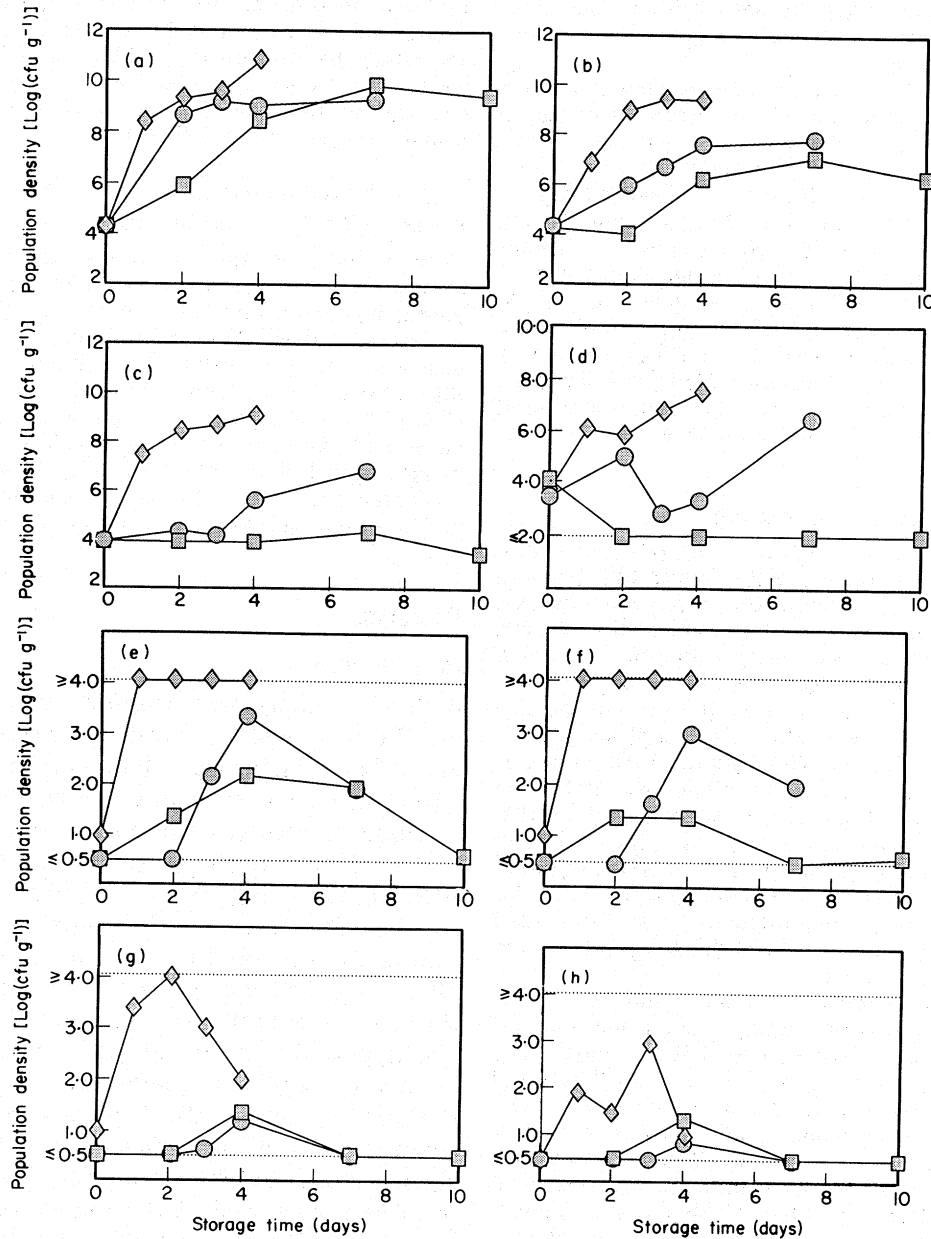
## **Results**

### *Raw shrimp*

A 28°C incubation in conjunction with an APC was used to maximize the recovery of bacteria through the inclusion of psychrotrophs that might not be enumerated at incubation temperatures of  $\geq$  35°C. This is apparent comparing the APC assays using 28 vs 37°C for raw shrimp [Fig. 1(a) and (b)] where the counts for the product held at 5 and 12°C were 1–2 log cycles higher using the lower incubation temperature. It is also apparent that an APC using a 28°C incubation would be ineffective in distinguishing temperature abused raw shrimp in that given sufficient time, the shrimp stored at 5°C attained the same level as that stored at the higher temperatures [Fig. 1(a)]. When the APC plates were incubated at 37°C [Fig. 1(b)], an approximate 2 log cycle differential was observed between the level

reached in the 19°C subsample vs 5 and 12°C. APCs with the plates being incubated at 42°C were used as a means of eliminating the majority of psychrotrophic species that would occur in

foods. In raw shrimp [Fig. 1(c)], use of the elevated incubation temperature provided a clear differential in the response of adequately refrigerated vs temperature abused product, with the



**Fig. 1.** The effect of incubating raw shrimp at 5, 12, and 19°C on levels detected using 28°C-APC (a), 37°C-APC (b), 42°C-APC (c), *Staphylococcus aureus* (d), presumptive coliform (e), confirmed coliform (f), thermal tolerant coliform (g), and *Escherichia coli* (h) assays. Dotted lines indicate upper or lower limits of detection.

counts remaining essentially constant in the 5°C subsample. *S. aureus* levels were clearly different in abused product compared to the 5°C counts [Fig. 1(d)]. The presumptive and confirmed coliform assays yielded a clear differential between the grossly abused and properly refrigerated shrimp, but results with the moderately abused product were less clearcut [Fig. 1(e,f)]. The results with the two tests were similar, although the levels in the 5°C samples were somewhat lower with the confirmed assay. The use of the elevated incubation temperature associated with thermal tolerant coliform test provided an even sharper differential between the 5 and 19°C; however, moderate abuse gave results similar to that observed with 5°C storage [Fig. 1(g)]. The levels of thermal tolerant coliforms in the 19°C sample fell after prolonged abuse, but this would likely have little practical significance since at that point the product was organoleptically objectionable. The ability of *E. coli* counts to distinguish gross abuse was less effective than the thermal tolerant coliform assay [Fig. 1(h)].

#### *Cooked shrimp*

No differential in the maximum bacterial population reached during adequate and abusive storage was observed with the 28°C-APC [Fig. 2(a)] and 37°C-APC [Fig. 2(b)] assays. However, the 42°C-APC yielded a clear differential of as much as 3–4 log cycles between the adequately stored product and both the moderately and grossly abused product, with the bacterial counts not exceeding  $10^6$  cfu g<sup>-1</sup> in the properly refrigerated product [Fig. 2(c)]. The *S. aureus* assay differentiated properly refrigerated and grossly abused product, but not moderately abused product [Fig. 2(d)]. The results of the presumptive [Fig. 2(e)] and confirmed [Fig. 2(f)] coliform assays

were similar, with the coliform levels in the moderately and grossly abused product rapidly increasing above the upper limit of detection employed in the study. The coliform levels in the properly refrigerated cooked shrimp actually fell for the first 4 days of storage, whereupon it regained its initial level. A clear differential between properly refrigerated and grossly abused product was apparent with the thermal tolerant coliform [Fig. 2(g)], and *E. coli* [Fig. 2(h)] assays; however, the levels were erratic when the product was held at 12°C.

#### *Lump crabmeat*

The 28°C-APC [Fig. 3(a)] was ineffective, and the 37°C-APC [Fig. 3(b)] and 42°C-APC [Fig. 3(c)] only differentiated grossly abused product. Grossly abused, but not moderately abused, product was readily differentiated by *S. aureus* counts [Fig. 3(d)]. Grossly abused product, and to a lesser extent moderately abused product, had somewhat elevated presumptive coliform levels, whereas coliforms were not detected in the sample stored at 5°C [Fig. 3(e)]. Differences in confirmed coliform counts between adequately refrigerated and abused crabmeat were minimal [Fig. 3(f)], and thermal tolerant coliforms [Fig. 3(g)] and *E. coli* [Fig. 3(h)] were not detected in the product.

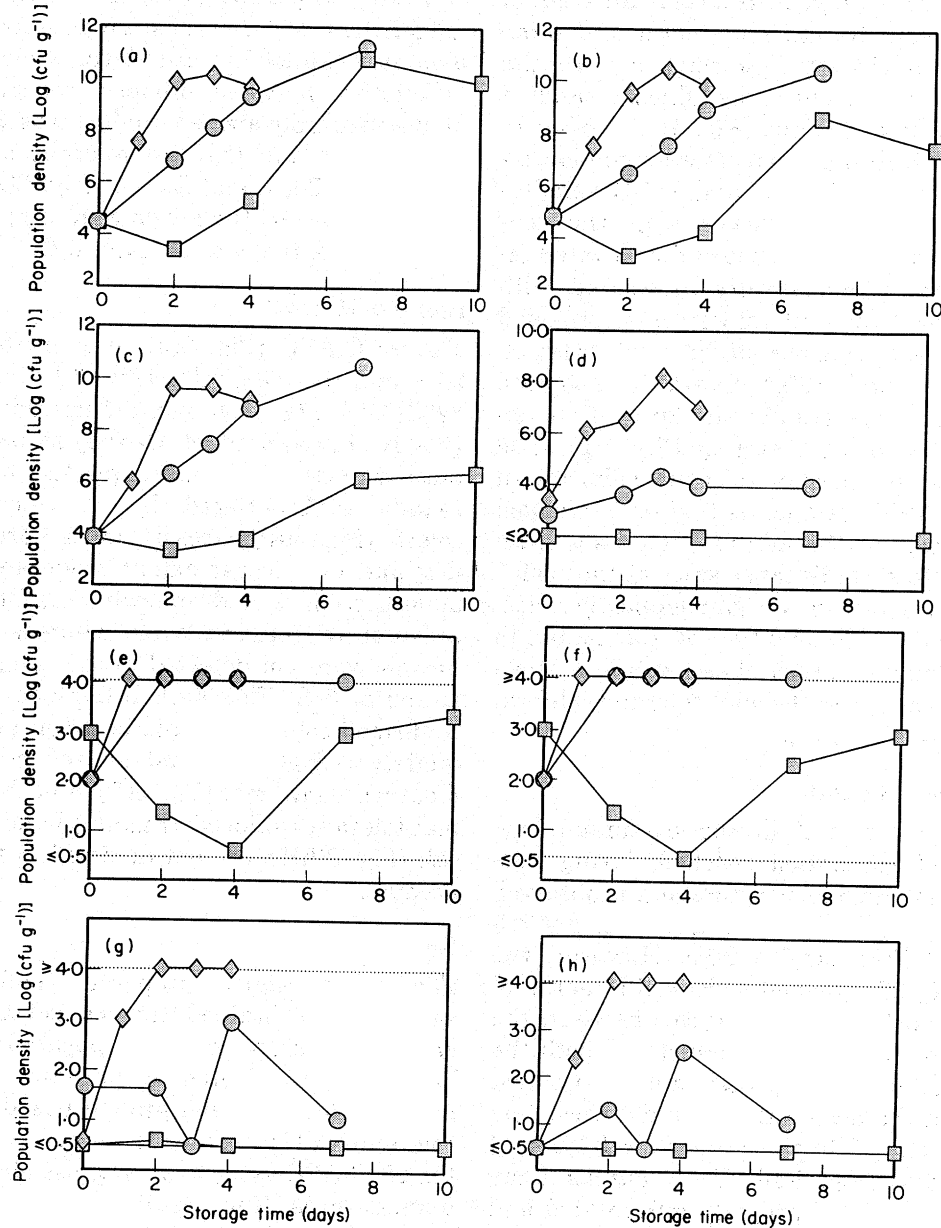
#### *Seafood salad*

The commercially prepared seafood salad sample obtained from a supermarket delicatessen counter appeared to have been formulated to minimize the potential for microbial growth based on the minimal increases observed with the 28°C-APC assay even at gross abuse temperatures [Fig. 4(a)]. In this instance, none of the indicator tests were effective either due to absence of the indicator or its inability to grow in the product [Figs 4(b–h)].

### Raw ground beef

There was no difference in the final bacterial levels reached in raw ground beef samples stored at the three temperatures using a 28°C-APC [Fig. 5(a)]. A

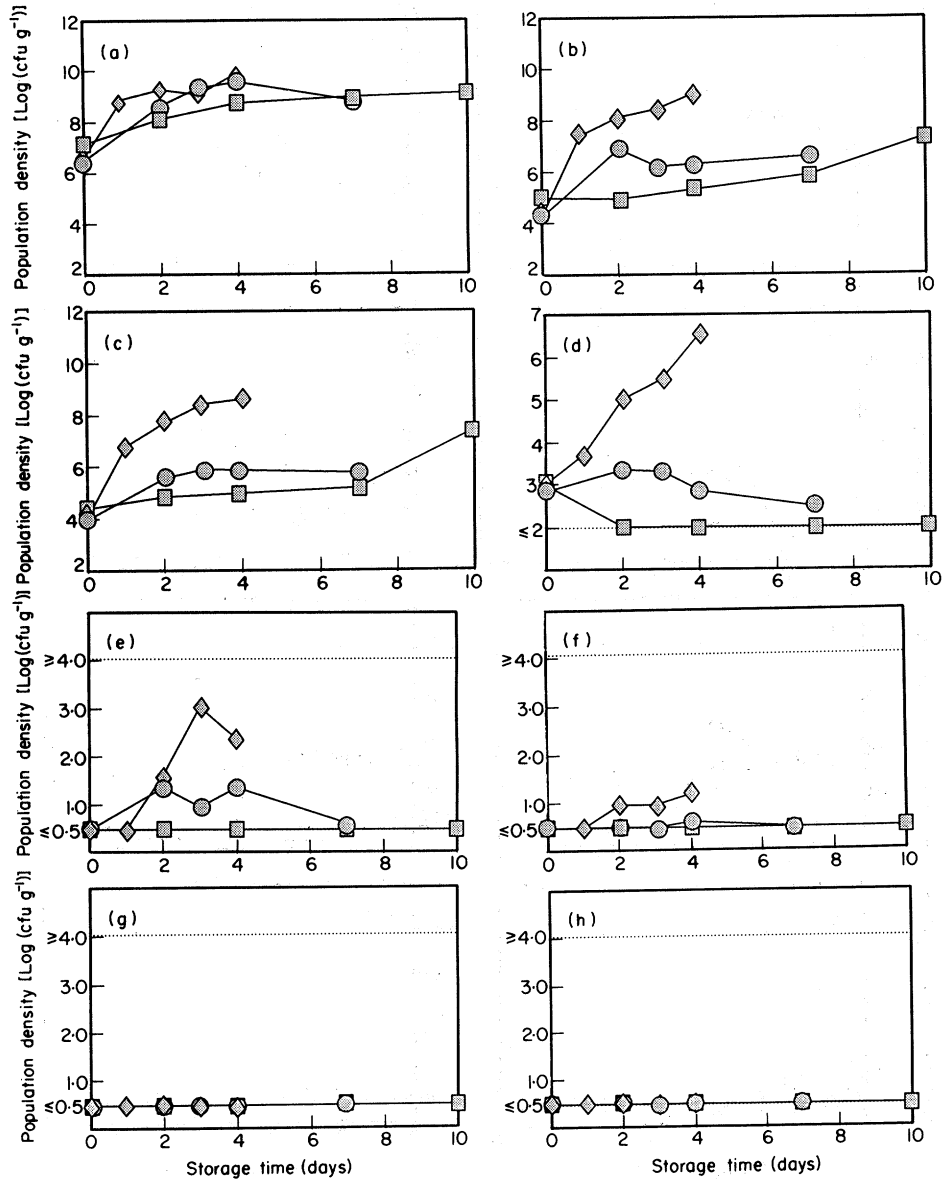
small differential was observed between abused and properly refrigerated samples with the 37°C-APC [Fig. 5(b)], and the 42°C-APC distinguishing readily both the moderately and grossly abused



**Fig. 2.** The effect of incubating cooked shrimp at 5, 12, and 19°C on levels detected using 28°C-APC (a), 37°C-APC (b), 42°C-APC (c), *Staphylococcus aureus* (d), presumptive coliform (e), confirmed coliform (f), thermal tolerant coliform (g) and *Escherichia coli* (h) assays. Dotted lines indicate upper or lower limits of detection.

samples [Fig. 5(c)]. A differential between *S. aureus* counts occurred between grossly abused and refrigerated samples; however, the response for moderately abused product was less clearcut

[Fig. 5(d)]. Growth of psychrotrophic coliforms was apparent with the presumptive and confirmed coliform assays [Figs 5(e,f)]; however, grossly abused and, to a lesser extent, moderately abused



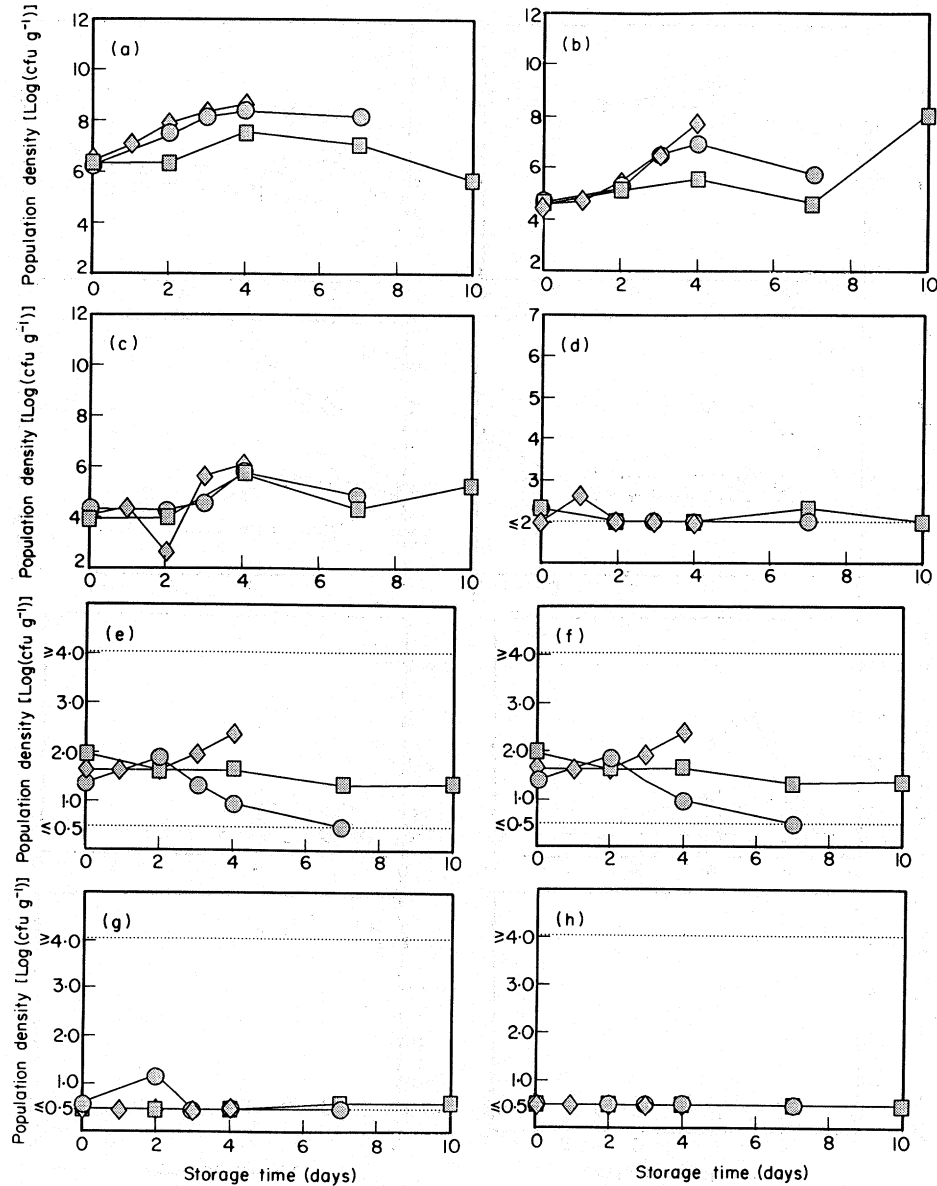
**Fig. 3.** The effect of incubating lump crabmeat (unpasteurized) at 5, 12, and 19°C on levels detected using 28°C-APC (a), 37°C-APC (b), 42°C-APC (c), *Staphylococcus aureus* (d) presumptive coliform (e), confirmed coliform (f) thermal tolerant coliform (g) and *Escherichia coli* (h) assays. Dotted lines indicate upper or lower limits of detection.

ground beef could be differentiated using assays for thermal tolerant coliforms and *E. coli* [Figs 5(g,h)].

#### Raw ground pork

The results observed with the raw pork

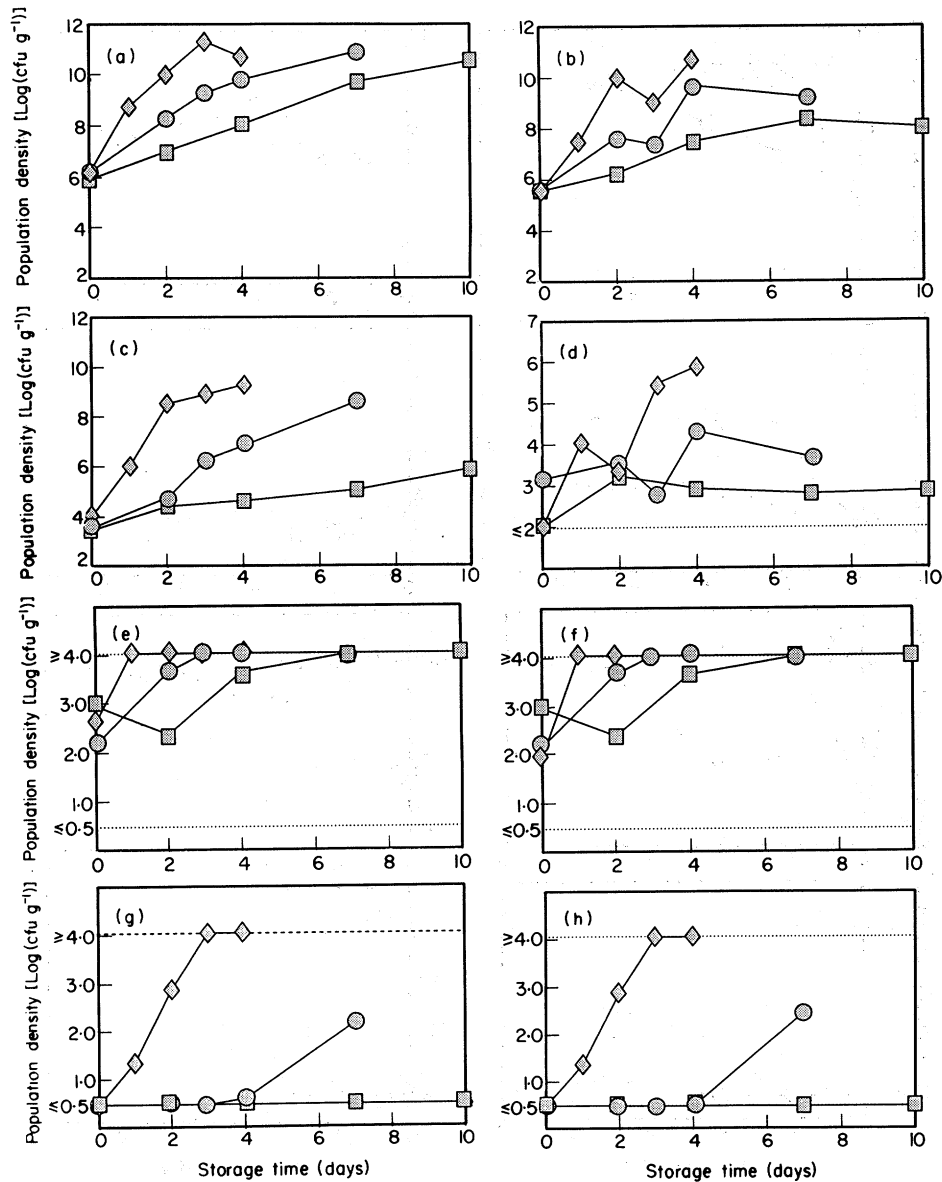
sample [Figs 6(a-h)] were largely similar to those for raw ground beef. The 42°C-APC was comparatively less effective due to a combination of a lower final level of organisms in the temperature abused samples and increases in the



**Fig. 4.** The effect of incubating seafood salad at 5, 12, and 19°C on levels detected using 28°C-APC (a), 37°C-APC (b), 42°C-APC (c), *Staphylococcus aureus* (d), presumptive coliform (e), confirmed coliform (f), thermal tolerant coliform (g) and *Escherichia coli* (h) assays. Dotted lines indicate upper or lower limits of detection.

number of bacteria capable of tolerating the elevated incubation temperature upon extended refrigerated storage [Fig. 6(c)]. A significant difference from raw ground beef was the lack of *S. au-*

*reus* growth in temperature abused raw ground pork [Fig. 6(d)]. Growth of psychrotrophic coliforms was again evident [Figs 6(e,f)], but temperature abused product could be differentiated to a de-



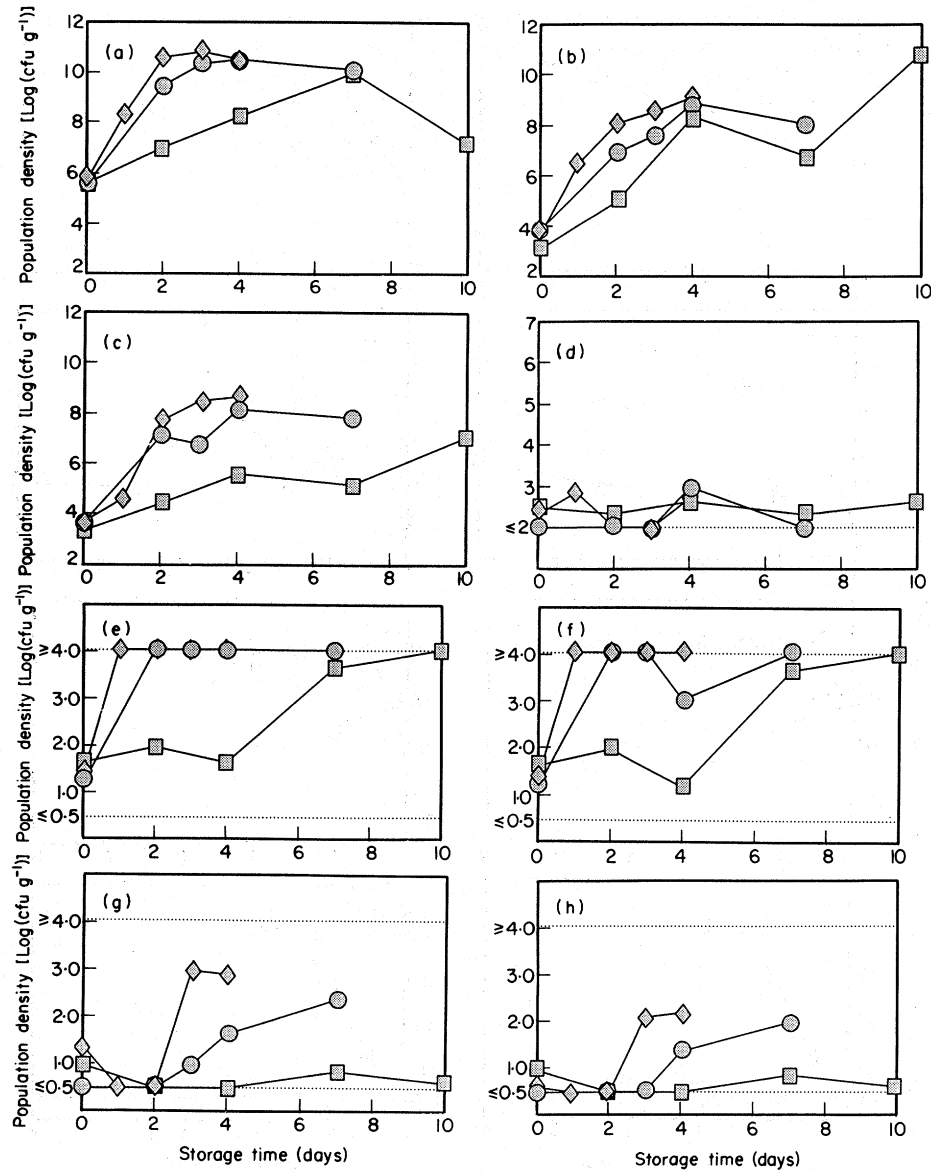
**Fig. 5.** The effect of incubating raw ground beef at 5 (■), 12 (●), and 19°C (◆) on levels detected using 28°C-APC (a), 37°C-APC (b), 42°C-APC (c), *Staphylococcus aureus* (d), presumptive coliform (e), confirmed coliform (f), thermal tolerant coliform (g) and *Escherichia coli* (h) assays. Dotted lines indicate upper or lower limits of detection.

gree based on increases in the number of thermal tolerant coliforms [Fig. 6(g)] and *E. coli* [Fig. 6(h)].

#### Sliced roast beef

Sliced roast beef obtained from the deli-

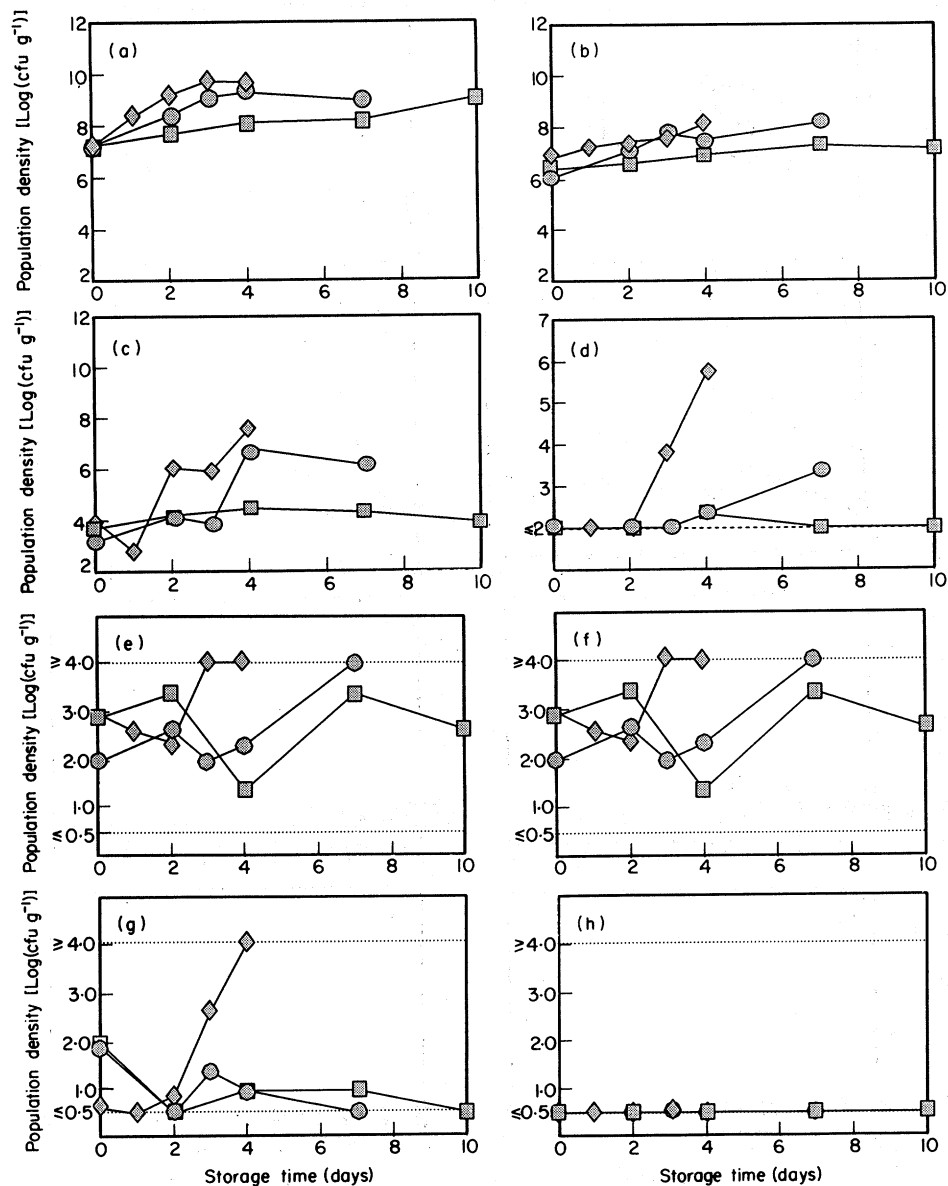
catesen section of a supermarket was used as an example of an uncured ready-to-eat meat product. The product had a rather high initial bacterial load, and temperature-abused product could not be differentiated using either 28°C-



**Fig. 6.** The effect of incubating raw ground pork at 5 (■), 12 (●), and 19°C (◆) on levels detected using 28°C-APC (a), 37°C-APC (b), 42°C-APC (c), *Staphylococcus aureus* (d), presumptive coliform (e), confirmed coliform (f) thermal tolerant coliform (g) and *Escherichia coli* (h) assays. Dotted lines indicate upper or lower limits of detection.

APC [Fig. 7(a)] or 37°C-APC [Fig. 7(b)] counts. The 42°C-APC counts increased 2–3 log cycles in temperature abused product, whereas the adequately refrigerated samples maintained their initial

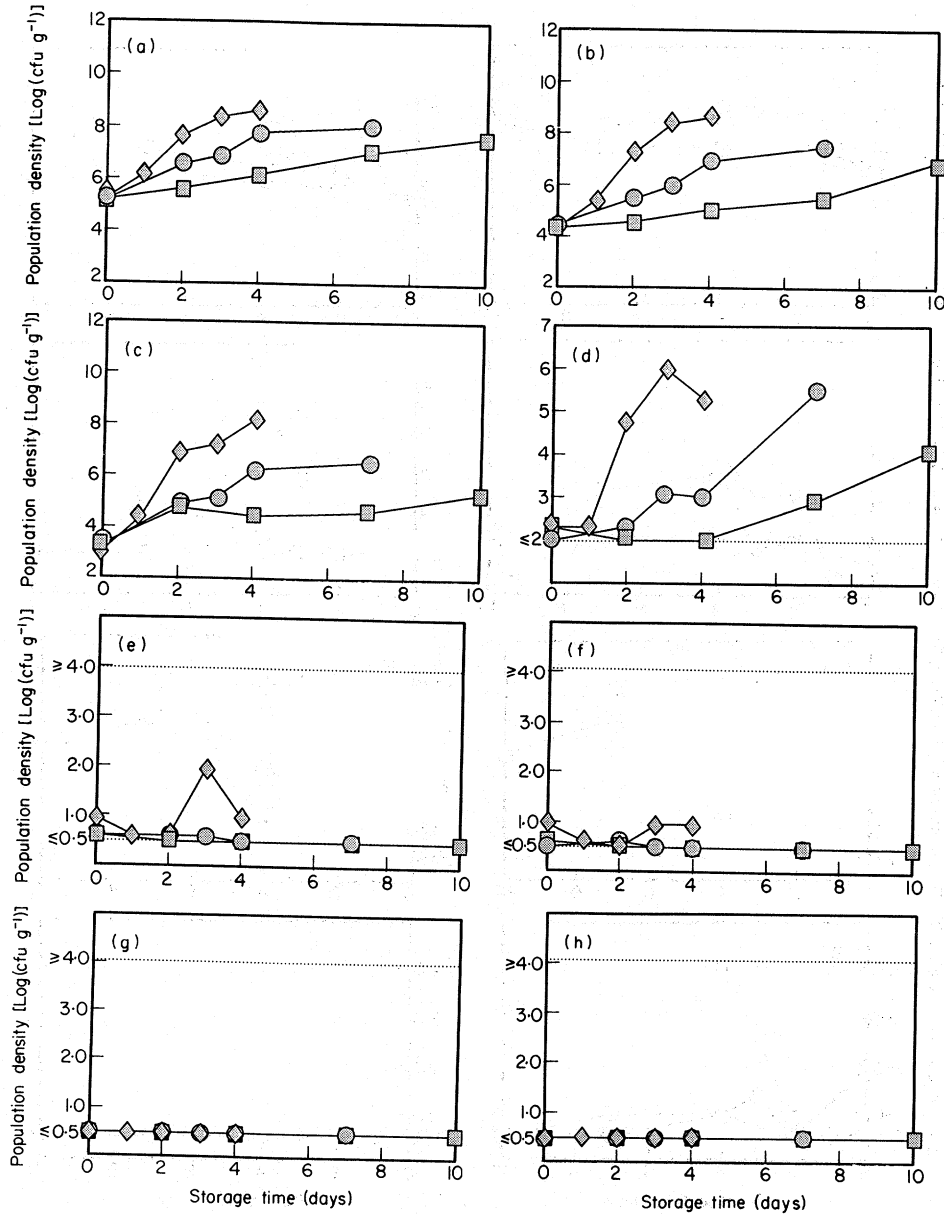
level over the course of 10 days of storage [Fig. 7(c)]. The levels of *S. aureus* remained unchanged in properly refrigerated product, but increased substantially in the grossly abused product [Fig.



**Fig. 7.** The effect of incubating sliced roast beef at 5 (■), 12 (●), and 19°C (◆) on levels detected using 28°C-APC (a), 37°C-APC (b), 42°C-APC (c), *Staphylococcus aureus* (d), presumptive coliform (e), confirmed coliform (f) thermal tolerant coliform (g) and *Escherichia coli* (h) assays. Dotted lines indicate upper or lower limits of detection.

7(d)]. Increases in *S. aureus* counts were minimal in the moderately abused product, and only occurred late in the storage period. The levels of coliforms [Fig. 7(e,f) eventually increased to above the

upper limit of detection ( $> 10^4 \text{ g}^{-1}$ ) in the abused samples, whereas the refrigerated product fluctuated around the initial level of approximately  $10^3 \text{ g}^{-1}$ . The initial level of thermal tolerant



**Fig. 8.** The effect of incubating sliced ham at 5 (■), 12 (●), and 19°C (◆) on levels detected using 28°C-APC (a), 37°C-APC (b), 42°C-APC (c), *Staphylococcus aureus* (d), presumptive coliform (e), confirmed coliform (f) thermal tolerant coliform (g) and *Escherichia coli* (h) assays. Dotted lines indicate upper or lower limits of detection.

coliforms was 1–2 log cycles lower and only the sample stored at 19°C increased over the storage regime [Fig. 7(g)]. The increased levels of thermal tolerant coliforms in the grossly abused product was not reflected in increased levels of *E. coli*, since none were detected [Fig. 7(h)].

#### *Sliced ham*

Differences in the 28°C-APC [Fig. 8(a)] and 37°C-APC [Fig. 8(b)] counts for properly and improperly stored products were too small to be effective for distinguishing abused sliced ham. However, the differential in counts was enhanced by increasing the incubation temperature to 42°C [Fig. 8(c)], particularly for the grossly abused product. Based on BPA counts, the levels of *S. aureus* rose quickly in the temperature abused product; however, increases were also noted in the properly refrigerated product [Fig. 8(d)]. Coliforms, thermal tolerant coliforms, and *E. coli* were present in very low numbers or were not detected, even after the extended storage at abuse temperatures [Fig. 8(e–g)].

#### *Raw ground chicken*

The 28°C-APC [Fig. 9(a)] was ineffective for distinguishing temperature abused raw ground chicken meat, but relatively small differences between the final bacteria levels reached by abused and properly refrigerated product were observed with 37°C-APC [Fig. 9(b)] and 42°C-APC [Fig. 9(c)]. A clear differential between adequately refrigerated and even moderately abused product was observed with *S. aureus* [Fig. 9(d)]. Initial coliform counts were relatively high, and quickly exceeded the upper limit of detection at all incubation temperatures [Fig. 9(e,f)]. The initial counts of thermal tolerant coliforms [Fig. 9(g)] and *E. coli* [Fig. 9(h)] were also relatively high, but particularly with *E. coli*, there

was a decline in the levels detected in the refrigerated samples.

#### *Sliced chicken roll*

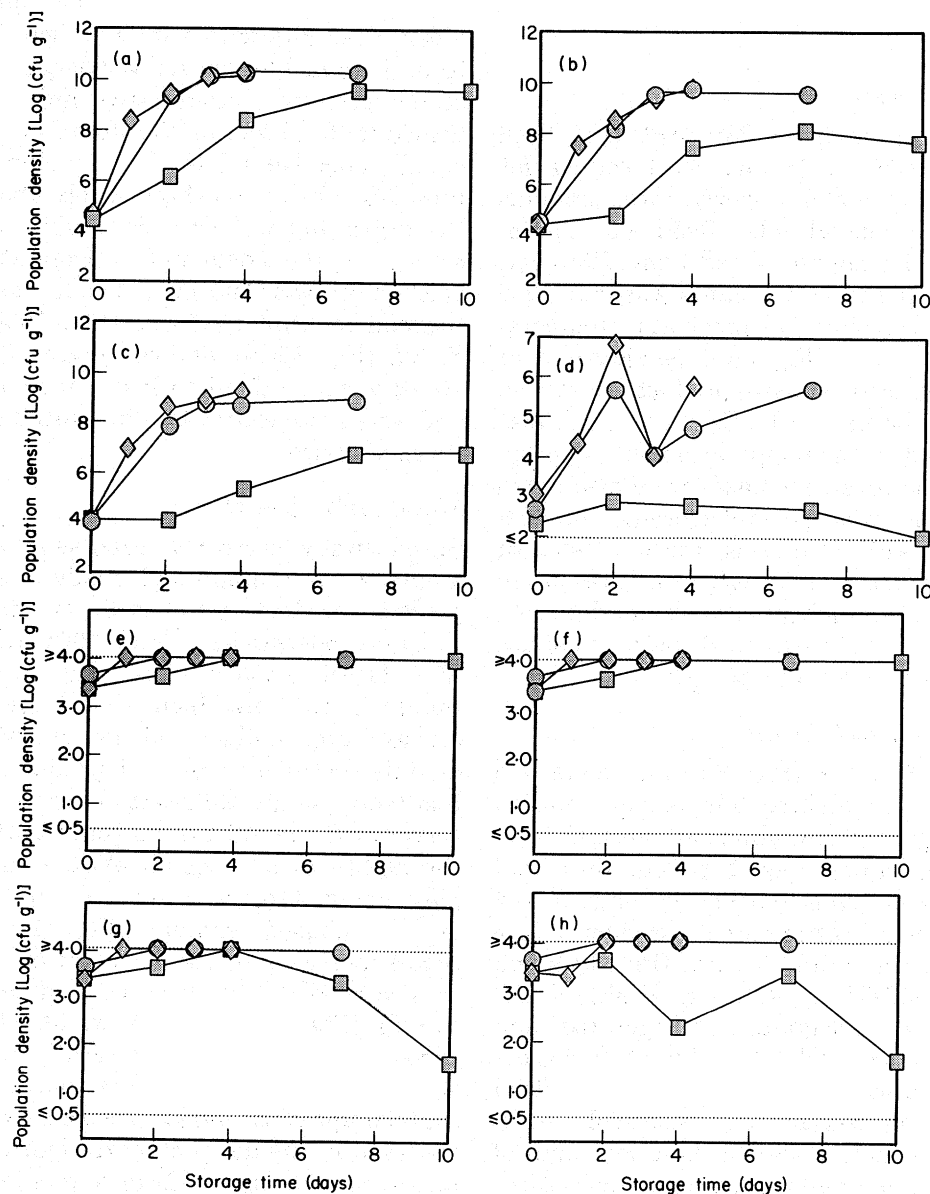
The 28°C-APC [Fig. 10(a)] was again ineffective, but both the 37°C-APC [Fig. 10(b)] and 42°C-APC [Fig. 10(c)] provided clear differences in the bacterial levels obtained for abused vs adequately stored product. Presumptive and confirmed coliform counts [Fig. 10(e,f)] were equally effective; the level in the abused products quickly reaching levels above the upper limit of detection, while increases in the adequately refrigerated chicken roll were minimal. The levels of thermal tolerant coliforms [Fig. 10(g)], *E. coli* [Fig. 10(h)], and *S. aureus* [Fig. 10(d)] were minimal to non-detectable, and were not effective for differentiating thermal abuse.

#### *Sliced turkey breast*

Sliced turkey breast was used as an example of non-comminuted, ready to eat poultry product purchased at delicatessen operations. Final 28°C-APC [Fig. 11(a)] and 37°C-APC [Fig. 11(b)] counts for the three incubation temperatures were similar, and did not adequately distinguish abused product. A relatively small differential was observed with the 42°C-APC counts, with the ability to distinguish abused samples being largely restricted to the grossly abused sample [Fig. 11(c)]. Small increases in the levels of *S. aureus* were noted in the grossly abused sample [Fig. 11(d)]; however, like the sliced ham sample [Fig. 8(d)], psychrotrophic growth of species that gave a presumptive *S. aureus* response was observed in the adequately refrigerated turkey breast. The coliform counts [Fig. 11(e,f)] remained low in the 5°C sample but increased sharply in the 19°C sample. The 12°C sample gave an intermediate response; however, the differential

between the moderately abused and adequately refrigerated turkey breast was sufficient to differentiate the two, particularly with the confirmed coliform test. The levels of thermal tolerant

coliforms [Fig. 11(g)] and *E. coli* [Fig. 11(h)] were extremely low even after extended storage, and no useful differences were noted between the samples.

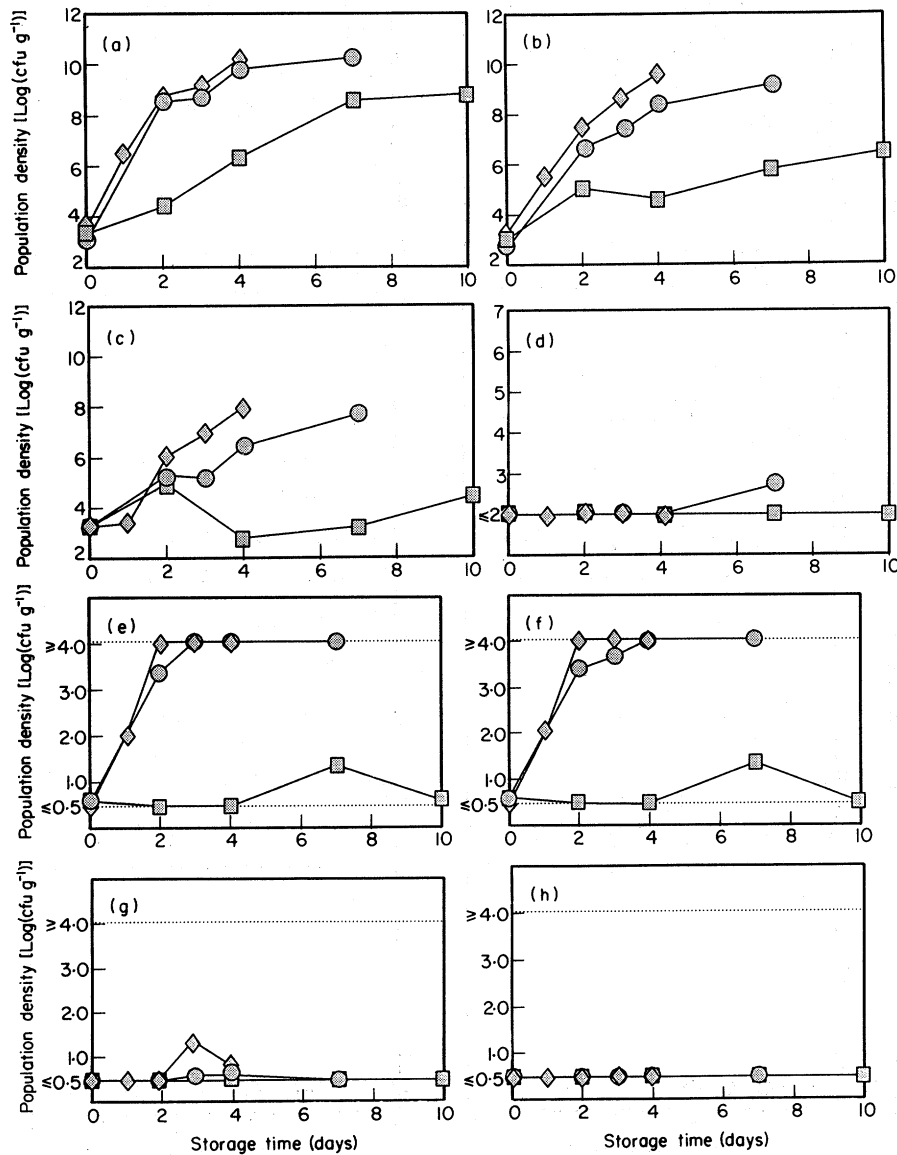


**Fig. 9.** The effect of incubating raw ground chicken at 5 (■), 12 (●), and 19°C (◆) on levels detected using 28°C-APC (a), 37°C-APC (b), 42°C-APC (c), *Staphylococcus aureus* (d), presumptive coliform (e), confirmed coliform (f) thermal tolerant coliform (g) and *Escherichia coli* (h) assays. Dotted lines indicate upper or lower limits of detection.

### Chicken salad

The response with the three APC incubation temperatures were similar, with minimal growth of bacteria at any incubation temperature, and no differences

between abused and refrigerated product being detected [Fig. 12(a-c)]. Coliforms [Fig. 12(e,f)], thermal tolerant coliforms [Fig. 12(g)], and *S. aureus* [Fig. 12(d)] declined over time at each storage

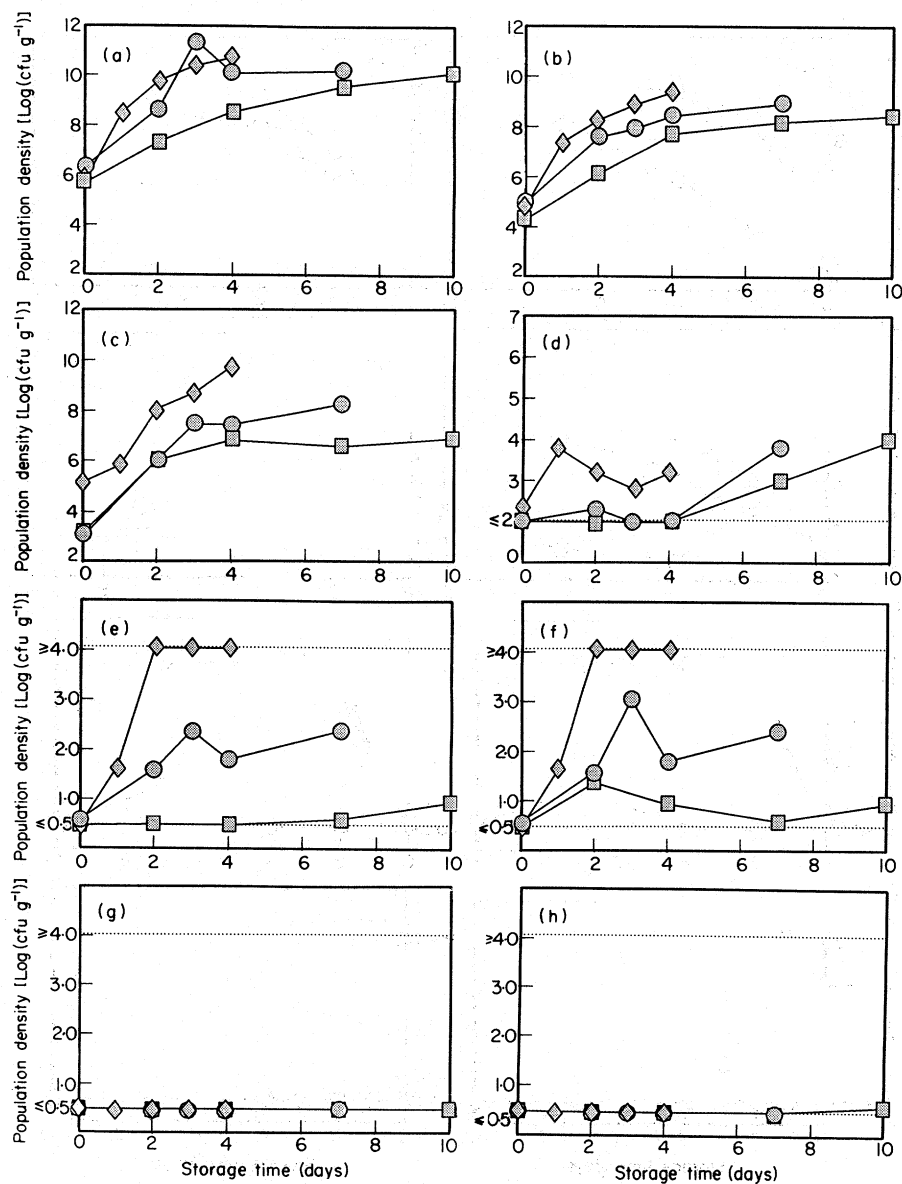


**Fig. 10.** The effect of incubating sliced chicken roll at 5 (■), 12 (●), and 19°C (◆) on levels detected using 28°C-APC (a), 37°C-APC (b), 42°C-APC (c), *Staphylococcus aureus* (d), presumptive coliform (e), confirmed coliform (f) thermal tolerant coliform (g) and *Escherichia coli* (h) assays. Dotted lines indicate upper or lower limits of detection.

temperature, and *E. coli* was not detected in the product [Fig. 12(h)]. This product also appears to have been formulated to retard bacterial growth, including having been acidified to a pH of approximately 4-7.

#### APCs at elevated temperatures

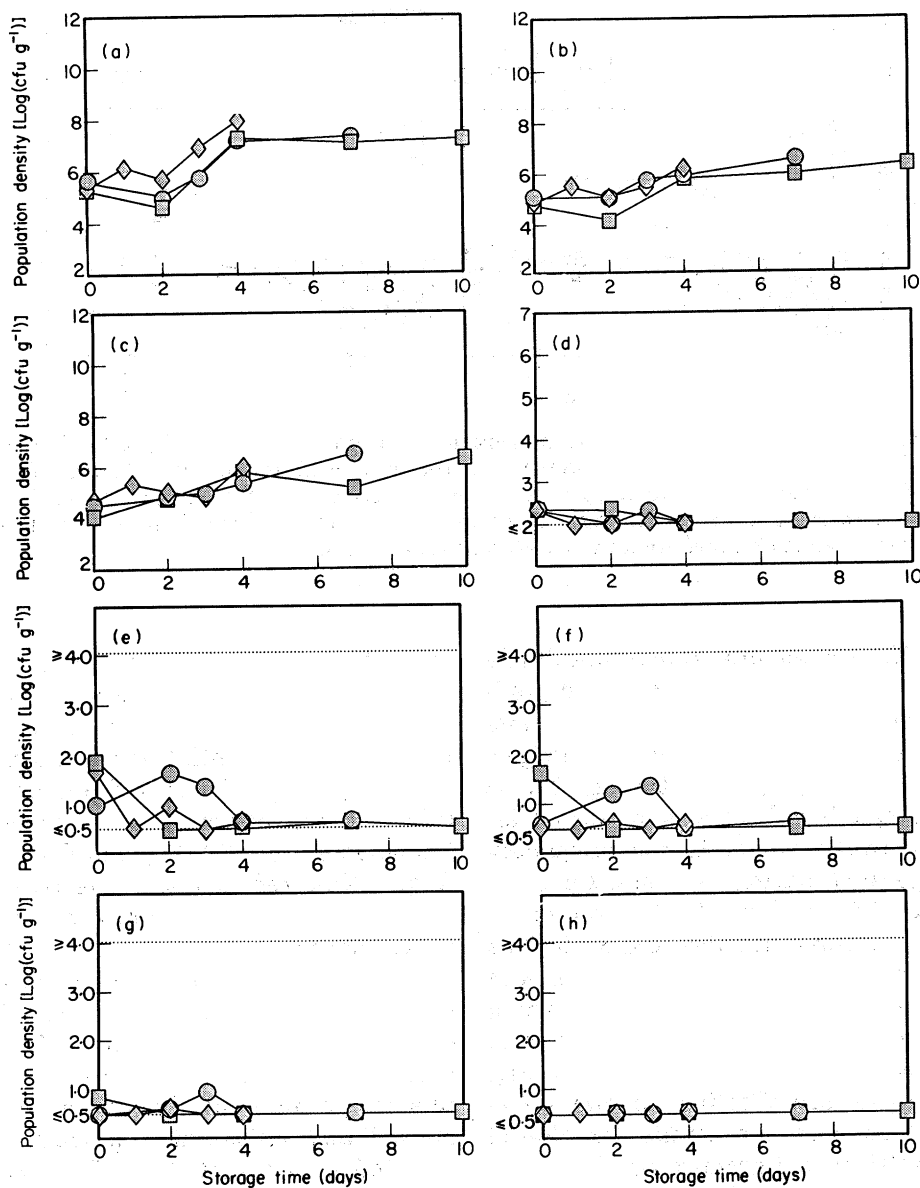
The data above indicated that one of the assays with potential for detecting temperature abuse in a number of products was an APC with the incubation temperature increased to 42°C. A set of sup-



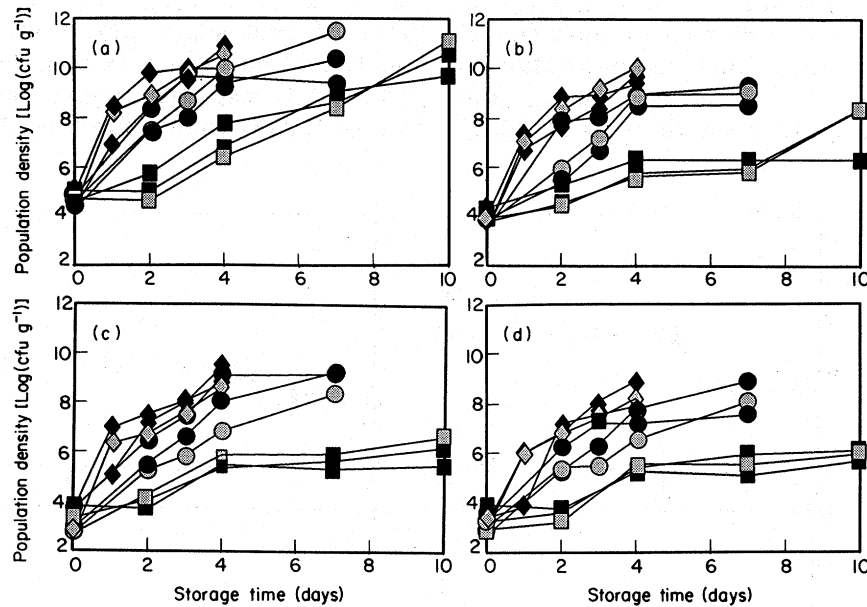
**Fig. 11.** The effect of incubating sliced turkey breast at 5 (■), 12 (●), and 19°C (◆) on levels detected using 28°C-APC (a), 37°C-APC (b), 42°C-APC (c), *Staphylococcus aureus* (d), presumptive coliform (e), confirmed coliform (f) thermal tolerant coliform (g) and *Escherichia coli* (h) assays. Dotted lines indicate upper or lower limits of detection.

plemental studies with raw and cooked shrimp was performed to determine if elevating the incubation temperature to 45 or 48°C could further enhance the differential between abused and refrigerated foods. Samples obtained from

three different supermarkets were examined as a means of assessing the consistency of response among sources. The 28°C-APC counts for raw shrimp



**Fig. 12.** The effect of incubating chicken salad at 5 (■), 12 (●), and 19°C (◆) on levels detected using 28°C-APC (a), 37°C-APC (b), 42°C-APC (c), *Staphylococcus aureus* (d), presumptive coliform (e), confirmed coliform (f) thermal tolerant coliform (g) and *Escherichia coli* (h) assays. Dotted lines indicate upper or lower limits of detection.



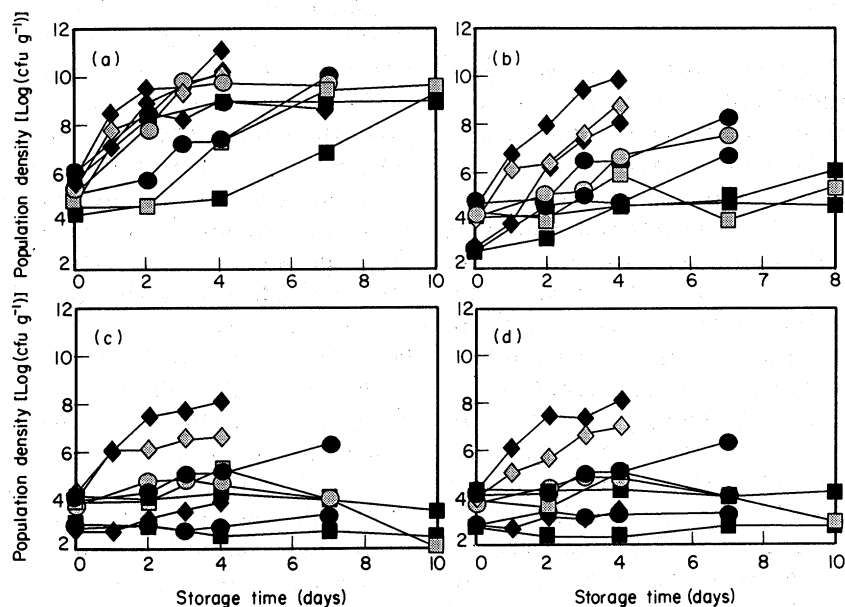
**Fig. 13.** The effect of incubating brain heart infusion agar plates at 28°C (a), 42°C (b), 45°C (c) and 48°C (d) on the ability of aerobic plate counts to differentiate raw shrimp that had been properly refrigerated (5°C; □) versus moderately (12°C; ●) and grossly (19°C; ◆) abused product. The three shadings among symbols indicate independent runs performed using raw shrimp obtained from three different supermarkets.

[Fig. 13(a)] again proved to be unsuitable for identifying temperature based product. The 42°C-APC [Fig. 13(b)] counts gave a clear difference between refrigerated and abused product through 7 days of storage, but there was an abrupt increase in the counts for the adequately refrigerated shrimp to approximately the level for moderately abused product for two of three samples on day 10. This did not occur when the APC plates were incubated at 45°C [Fig. 13(c)], which gave a good differential for detection of temperature abuse. The 48°C-APC [Fig. 13(d)] was also effective, but the differential between the counts for abused and refrigerated product was less than that observed with the 45°C-APC.

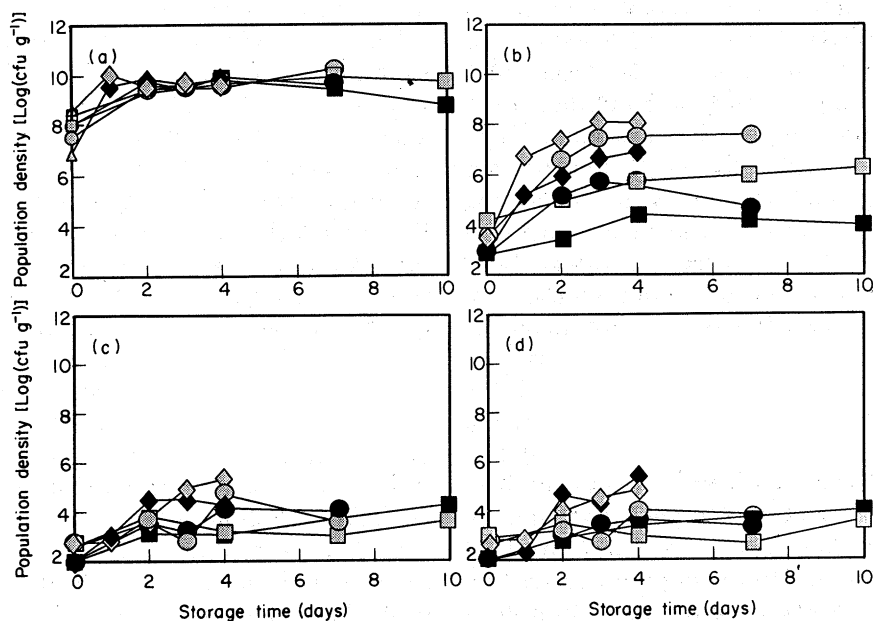
The three sets of 28°C-APC [Fig. 14(a)] and 42°C-APC [Fig. 14(b)] results for cooked shrimp were similar to those

presented previously [Fig. 2(a,c)] with the lower incubation temperature being ineffective and the higher temperature providing a differential between abused and adequately refrigerated product. In this instance, elevating the incubation temperature to 45°C [Fig. 14(c)] or 48°C [Fig. 14(d)] reduced the effectiveness for differentiating moderately abused cooked shrimp.

Two additional sets of cooked shrimp samples were overtly spoiled at the time of purchase, having initial 28°C-APC counts > 10<sup>7</sup> cfu g<sup>-1</sup> [Fig. 15(a)]. Even in this case, a differential between grossly abused and refrigerated product was detected with a 42°C-APC [Fig. 15(b)]. This difference was not observed when the incubation temperature was increased to 45°C [Fig. 15(c)] or 48°C [Fig. 15(d)].



**Fig. 14.** The effect of incubating brain heart infusion agar plates at 28°C (a), 42°C (b), 45°C (c), and 48°C (d) on the ability of aerobic plate counts to differentiate cooked shrimp that had been properly refrigerated (5°C; ■) versus moderately (12°C; ●) and grossly (19°C; ◆) abused product. The three shadings among symbols indicate independent runs performed using cooked shrimp obtained from three different supermarkets.



**Fig. 15.** The effect of high initial counts ( $\geq 10^7$  cfu g<sup>-1</sup>) on the ability of aerobic plate counts at 28°C (a), 42°C (b), 45°C (c), and 48°C (d) to differentiate cooked shrimp that had been properly refrigerated (5°C; ■) versus moderately (12°C; ●) and grossly (19°C; ◆) abused product. The two shadings among symbols indicate independent runs performed using cooked shrimp obtained from two different supermarkets.

## Discussion

Though they may enter the marketplace with a significant level of bacteria, typically refrigerated foods of animal origin have low levels of bacterial contamination at some point in their production. In the case of raw meats and poultry, it is generally assumed that the interior of muscle tissue has few, if any, bacteria, and that the surface of these products becomes contaminated during slaughter and subsequent handling. Similarly, it is assumed that immediately after capture, the bacterial levels in muscle tissue of fin fish and crustaceans taken from non-polluted waters will be low. (Raw molluscan shellfish represent a special case since the entire animal, including the intestinal tract, may be consumed.) The manufacture of most ready-to-eat products includes a lethal cooking step which helps ensure the inactivation of vegetative bacterial cells. Accordingly, the microbial flora of the product would be expected to reflect the organisms that are re-introduced after the cooking step due to handling, cross-contamination, etc. For example, Hackney et al. (1980) demonstrated that the cooking step in blue crab processing greatly reduced in the initial bacterial load. However, this advantage was quickly lost due to recontamination associated with the refuse containers for picking debris, insects, and cross contamination from live crab carts. Likewise, Ridley and Slabyj (1978) reported that after a significant reduction in bacterial levels as a result of cooking, bacterial loads in cooked peeled shrimp return to their initial levels as a result of recontamination during post-processing inspection and grading. The recontamination process resulted in an apparent shift in the microbial flora from psychrotrophic to mesophilic, including the introduction of *S. aureus*.

Assuming that initial bacterial levels in raw and cooked products are low and the extent of contamination/recontamination is controlled to a reasonable degree, then the number of bacteria present in the food during storage will be a function of growth of bacterial species, which in turn will be dependent primarily on the storage temperature. Temperature will affect both the rate of growth and the composition of the microbial flora. Incubating foods at low temperatures ( $\leq 5^{\circ}\text{C}$ ) will select for psychrotrophic species and prevent the growth of mesophiles. Abuse temperatures will permit growth of both psychrotrophs and mesophiles, with the latter being favoured as temperatures are increased. Ultimately, incubation temperatures will be reached that permit the growth of mesophiles, but not psychrotrophs. This differential response to temperature is the underlying basis that explains many of the results observed in the current study.

The three APC assays provide a good example of the impact of incubation temperature. The  $28^{\circ}\text{C}$ -APC would be expected to detect both psychrotrophs and mesophiles, with counts increasing if either grew. Thus, it would not be likely that this assay would differentiate growth in response to abuse conditions as compared with the normal growth of the microbial flora, i.e. it would not be able to distinguish if a product had been temperature abused or simply stored under adequate refrigeration for an extended period. This was in fact the case, with the inability of the  $28^{\circ}\text{C}$ -APC to differentiate abused products being the only response that was consistent with all of the foods examined. Elevating the incubation temperature of the APC plates to  $42^{\circ}\text{C}$  would be expected to prevent the growth of most psychrotrophic bacteria and only permit the growth of mesophiles. Thus, if a

product was maintained at refrigeration temperatures, the normal increase in the psychrotrophic microbial flora should not be reflected in the 42°C-APC counts. Most foods examined showed a clear differential in the 42°C-APC levels reached by abused vs refrigerated products, although the extent of the differential varied. As expected, the assay tended to perform better for differentiating products that had been grossly abused (19°C samples). It appears that there can be products where a significant portion of the population can grow at both 5 and 42°C, a characteristic that would confound the basis of the assay. As in the case of two of the raw shrimp samples (Fig. 14), further increases in incubation temperatures might eliminate the problem. The 42°C-APC assay was ineffective with two foods, seafood salad (Fig. 4) and chicken salad (Fig. 12). However, it was apparent that these products were formulated to prevent microbial growth. Thus, the underlying assumption that storage temperature was the primary factor limiting microbial growth was not valid with those foods, demonstrating the importance of that assumption. The 37°C-APC assay was effective with a limited number of foods, but overall it appeared that temperature was insufficient to differentiate psychrotrophs and mesophiles.

The usefulness of the assays for the different classes of coliforms varied substantially among the products tested, although a number of general observations can be made. In raw products [shrimp (Fig. 1), ground beef (Fig. 5), ground pork (Fig. 6), and ground chicken (Fig. 9)], the presumptive and confirmed coliform tests were ineffective due to the growth of psychrotrophic coliforms. Incorporation of an incubation step at an elevated temperature (45.5°C) suppressed the psychrotrophs, and resulted in the thermal tolerant col-

iform and *E. coli* assays being able to differentiate at least gross temperature abuse. These observations support the conclusion of Newton (1979) who suggested that the coliform test was not an effective indicator in raw meats, but that the fecal (thermal tolerant) coliform test had potential.

In cooked ready-to-eat products the effectiveness of the presumptive and confirmed coliform assays appeared to be dependent on whether the food product supported coliform growth. Three products, seafood salad (Fig. 4), chicken salad (Fig. 12), and sliced ham (Fig. 8), did not support the growth of coliforms even in the grossly abused product. The lack of coliform growth on sliced ham may reflect the use of salt and other curing agents in its formulation. With the possible exception of the lump crabmeat (Fig. 3), the presumptive and confirmed coliform tests were able to distinguish temperature abuse in the ready-to-eat products [shrimp (Fig. 2), roast beef (Fig. 7) chicken roll (Fig. 10) and turkey breast (Fig. 11)] that would support coliform growth, suggesting that initial populations of psychrotrophic coliforms were eliminated by the cooking step and subsequent recontamination was associated with mesophilic coliforms. Cooked shrimp (Fig. 2) and roast beef (Fig. 7), were the only ready-to-eat products where the thermal tolerant coliform assay differentiated abused products, and this was limited to gross abuse. This was due to either the inability of the food to support the growth of the micro-organisms or the lack of viable thermal tolerant coliforms in the product. The *E. coli* test was even less effective; the species was consistently absent in these products. While coliforms may be a common recontaminant in ready-to-eat products, this does not appear to be the case for thermal tolerant coliforms including *E. coli*.

The results with the presumptive and confirmed coliform assays were consistently similar. Likewise, the results with the thermal tolerant coliform and *E. coli* assays had a high degree of agreement. This suggests that if one were employing these assays for detecting temperature abuse, it may be sufficient to limit testing to the presumptive coliform or thermal tolerant coliform assays, and not extend the duration to determine levels of confirmed coliforms or *E. coli*, respectively.

While *S. aureus* may be associated with other sources (e.g. raw milk from mastitic cows), it is generally indicative of handling by humans. The microorganism's minimum growth temperature is approximately 10°C which may account for this assay being effective for detecting gross abuse but not moderate abuse in several foods. The assay was effective to varying degrees in three of the four raw products; with the organism unexpectedly not growing at gross abuse temperatures in raw ground pork (Fig. 6). The responses in ready-to-eat products were generally restricted to detection of gross abuse. As with the other assays, monitoring *S. aureus* levels is not effective for foods (seafood and chicken salads) that have been formulated such that temperature is no longer the primary limiting factor for growth. The assay was particularly effective in sliced hams where the added salt would tend to select for the growth of halotolerant species. However, this was confounded by the apparent psychrotrophic growth of *S. aureus* in the adequately refrigerated sample. It is likely that this was actually an interfering psychrotrophic enterococci, micrococci, other psychrotrophic species that can give false results on Baird-Parker agar. Use of this assay as an indicator test for detecting temperature abuse may require employing a more selective medium. An-

other alternative may be to elevate the temperature at which the plates are incubated to retard the growth of confounding species.

The supplemental studies with raw and cooked shrimp suggest that incubation temperatures can be selected to optimize the ability of an assay to differentiate abused and adequately refrigerated product (Figs 13 and 14). It also appears that appropriately selected indicator assays may be effective even in product that is approaching the end of its refrigerated shelf-life, when there are elevated levels of psychrotrophs (Fig. 15). The large differential between the 28°C-APC and 42°C-APC assays observed with the 0 h samples of the two poor quality batches of shrimp [Fig. 15(a,b)] suggest that these samples were spoiled due to extended refrigerator storage, not temperature abuse.

Since most of the data represent single samples obtained from a single retail outlet, the current study could not be analyzed statistically and must be considered preliminary in relation to making any recommendations. However, the similarities noted among the various shrimp samples that were collected at different times from different locations suggest that there are microbiological responses that are common for a product. Further, the study does clearly demonstrate that there are refrigerated food products of animal origin for which it should be feasible to select and use a common microbiological indicator test(s) or simple modification of a test to assess if there had been temperature abuse. Confirmation of this supposition will require acquisition of sufficient data to adequately categorize a number of products' microbiological responses to temperature abuse. These data could then serve as the scientific basis for establishing meaningful microbiological criteria for those products. However, the

current results also emphasize the importance of selecting indicator tests on a product-by-product basis. No single assay was effective for the twelve foods tested in the current study. Establishing microbiological criteria based on an indicator test without sufficient evaluation of the microbiological characteristics of individual products is likely to result in an ineffective criterion. While indicator assays have their limitations, understanding the assumptions under-

lying the tests allows one to select and use them both appropriately and effectively.

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